Evaluation of Anti-oxidant Activity Levels among Solanaceae Germplasm

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ABSTRACT

Improvement of tomato and pepper for antioxidant activity (AOA) could potentially benefit human health in many countries. We evaluated diverse entries of tomato (n=53) and pepper (n=46) for AOA and antioxidants (AO) for two years. Ample genetic variation was detected within both tomato and pepper to improve individual AO and AOA. Tomato entry means over years for inhibition of lipid peroxidation (ILP) ranged from 1.8–5.8 Trolox equivalent (TE) μmol · g^{-1} (fresh weight basis) with an overall mean of 3.97 TE μmol · g^{-1} and anti-radical power (ARP) values over years ranged from 2.5–6.8 TE μmol · g^{-1} with an overall mean of 4.11 TE μmol · g^{-1}. Entry means for lycopene ranged from 0.04–23.09 μmol · $100g^{-1}$ with an overall mean of 7.12 μg ·$100g^{-1}$. In general, means of the *L. pimpinellifolium* entries were significantly greater than *L. esculentum* for ARP, ILP, lycopene, vitamin C, phenolics, and soluble solids contents. Among the traits evaluated, total phenolics content showed the highest correlation with ARP (r=0.90**) and ILP (r=0.83**). Fruit size was negatively correlated with both ARP (r= -0.74**) and ILP (r= -0.71**). Capsicum entry means over years for ILP ranged from 13.0–61.8 TE μmol · g^{-1} (dry weight basis) with an overall mean of 27.4 TE μmol · g^{-1}. Means of brown-fruited entries were significantly higher than the mean of red-fruited entries for capsanthin, zeaxanthin, cryptoxanthin, ß-carotene, α-tocopherol and γ-tocopherol. We found a significant positive correlations between ILP and vitamin C (r=0.86**) and ILP and total phenolics content (r=0.86**).

Key words: Carotenoids, Phenolics, Tomato, Pepper.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum*) comprising both pungent and sweet types, are among the most widely grown and consumed vegetables in the world. In terms of human health, tomato fruit and pepper fruit contain significant amounts of vitamins and antioxidant phytochemicals, including carotenoids, vitamins C and E, and phenolics. Carotenoids β-carotene and ß-cryptoxanthin both possess provitamin A activity and also function to help prevent and neutralize free radical chain reactions and ascorbic acid is an effective scavenger of superoxide, hydrogen peroxide, singlet oxygen and other free radicals (Clevidence *et al.*, 1997). Lycopene, the major carotenoid in tomato

fruit, is a powerful AO and has attracted much attention because of the linkage between lycopene-rich diets and lower risks of certain cancers, heart disease, and age-related diseases (Clinton, 1998).

Tomato and pepper genotypes are diverse in the types and concentrations of carotenoids (Stommel and Haynes, 1994; Wall *et. al.* 2001), levels of ascorbic acid, and phenolics (Abushita *et al.*, 1997). Selection for higher concentrations of particular AO is one strategy to improve tomato and pepper for overall antioxidant activity (AOA) (Tucker, 2003) Because AO in plants usually occur in mixtures, overall AOA may be greater or lesser than the sum of individual AO due to synergies or antagonisms (Arnao *et al.*, 2001; Halliwell *et al.*, 1995; Kochian and Gavin, 1999; Stahl *et al.*, 1998), or bioactivity of unknown AO (Arnao *et al.*, 2001). Because tomato and pepper are consumed daily in many countries, production and consumption of tomato and pepper varieties of higher AOA could potentially contribute to better human health worldwide. The objectives of this study were to assess genetic diversity within *L. esculentum* and *C. annuum* for AO and AOA.

MATERIALS AND METHODS

Entries included 53 tomato and 46 pepper representing diverse origins, fruit colors and sizes. Tomato and pepper trials were grown two years in fields of AVRDC-the World Vegetable Center from October 2001 – March 2002 (2002 season) and October 2002 – March 2003 (2003 season). Mean maximum and minimum temperatures during this period range from 22-30 °C and 12-22 °C. Average monthly rainfall is < 50 mm. Entries were replicated twice and arranged in a randomized complete block design. Plots included five plants in the 2002 season and ten plants in the 2003 season. Fully mature fruit were harvested from each plot once when most plants within plots had ripe fruit. Fruit were taken the same day to the laboratory samples were prepared for measurement of either fruit qualities, individual AO, or AOA. Samples for measurement of AO or AOA were stored in sealed bags at –70 °C until analysis time.

Entries were analyzed for AOA by anti-radical power (ARP) and/or inhibition of lipid peroxidation (ILP) methods as described in Hanson *et al.* (2004a) and Hanson *et al.* (2004b). ARP measures the capacity of different components to scavenge the ABTS radical cation as compared to the standard antioxidant Trolox (0 – 4 mM) in a dose response curve. Trolox is a vitamin E analog. The AOA of a sample for the ARP assay was measured within the linear relationship of concentration vs. optical density decrease, and presented as Trolox equivalent (TE) in μmol·g^{-1} fresh weight basis for tomato and TE μmol·g^{-1} dry weight basis for pepper. The ability of AO samples to inhibit lipid peroxidation at pH 7 was tested using linoleic acid as a substrate and AAPH as a free radical generator. The absorbance difference of the reaction without linoleic acid and with linoleic acid was represented as 100% of inhibition. Trolox (0 – 4 mM) was used as the antioxidant standard and AOA was expressed as TE in μmol·g^{-1} tomato fruit (fresh weight basis). Carotenoids, total ascorbic acid, and total soluble phenolics were determined as described in Hanson *et al.* (2004a) and Hanson *et al.* (2004b).

Variables were subjected to analysis of variance (ANOVA) over years with the SAS General Linear Model (GLM) procedure

(SAS Institute, Inc., Cary, N.C.) where year was regarded as a random effect, entry as a fixed effect, and the year x entry interaction mean square was used to test the significance of the entry mean square and linear contrasts.

RESULTS

Tomato

Tomato entry means over years in for ILP ranged from 1.8 – 5.8 TE μmol·g^{-1} and ARP values over years ranged from 2.5 – 6.8 TE μmol·g^{-1} (Table 1). The three *L. pimpinellifolium* accessions LA1582, LA1586 and LA1593 ranked first, second, and third highest, respectively for ARP, and second, eleventh and seventh, respectively for ILP. In general, small-fruited *L. esculentum* entries (<31g) produced significantly higher AOA than the medium and large-fruited entries. LA1315, LA1385, LA1455 and LA1456, all of which are small-fruited and classified as *L. esculentum* var. *cerasiforme* or South American landraces, demonstrated the highest levels of AOA among *L. esculentum* entries. All medium, large, and very large-fruited entries showed low or moderate AOA. For most entries, lycopene was the major carotenoid although the range of values was wide (0.04 to 23.09 mg·100 g^{-1} (fresh mass basis)). The three *L. pimpinellifolium* entries formed a distinct group of means that were significantly larger than all the *L. esculentum* entries. The *L. esculentum* entries producing high lycopene content included T5019 and T4065 containing *dg* + *og*^{c} and *hp* + *og*^{c} genes, respectively, as well as LA1315. CLN1314G and 97L63 containing the Beta gene showed low lycopene density and the highest β-carotene levels (3.73 and 3.38 mg·100 g^{-1}, respectively) compared to an average β-carotene content of 0.61 mg·100 g^{-1} for the red-fruited entries. Entry means over years for phenolics content ranged from 56-156 mg·100 g^{-1} with an overall mean of 90 mg·100 g^{-1}. Entry means over years for ascorbic acid content ranged from 12-40 mg·100 g^{-1}. Almost all *L. esculentum* entries of relatively high phenolics content were small-fruited and means of almost all medium or large-fruited entries fell below 90 mg·100 g^{-1}. Like phenolics content, entries producing the highest ascorbic acid content included the *L. pimpinellifolium* and small-fruited *L. esculentum* such as LA1455.

Pepper

ILP values ranged from 13.0-61.8 μmol·g^{-1} (DW basis) (Table 2). CAP178/77, the accession with the highest ILP value, also ranked first among entries for total phenolics content and second highest for ascorbic acid content. Over entries, capsanthin was the major carotenoid, accounting for about two-thirds of the total carotenoids. However, the range of entry means for capsanthin was vast (0-1585 μg·g^{-1}). ß-carotene entry means ranged from 2-292 μg·g^{-1}. The brown-fruited entries, in general, produced more carotenoids than red-fruited entries. Entries 'Guajillo Ancho' and 'Verdano Poblano TY' ranked first and second highest, respectively, for capsanthin content over years and ranked among the top five for the other carotenoids. Ascorbic acid values ranged from 2.3-21.0 mg·g^{-1}. Simonne *et al.* (1997) reported higher ascorbic acid content in brown bell peppers compared to other colored bell fruit and our results showed about 19% higher ascorbic acid content in brown- versus red- fruited accessions. Orange-fruited accessions, as a group, developed the highest ascorbic acid levels with about 48% greater than the mean

Table 1. Selected *Lycopersicon esculentum and L. pimpinellifolium* entries evaluated for antioxidant activity, antioxidants, and fruit qualities, AVRDC-the World Vegetable Center Taiwan, 2001-2002 and 2002-2003. Values are fresh weight means (± SE) averaged over years.

Entry name (carotenoid gene)	Species	Fruit size category	Antioxidant activity ILP	Antioxidant activity ARP	Antioxidants Lycopene	Antioxidants ß-carotene	Antioxidants Phenolics	Antioxidants Ascorbic acid	Fruit qualities Color	Fruit qualities Soluble solids
			TE (µmol•g^{-1})		(mg•100 g^{-1})				(a/b)	(%)
LA 1582	L.p.	very small	5.77 ± 0.07	6.84 ± 0.93	23.09 ± 0.67	0.88 ± 0.18	156 ± 22	39.7 ± 5.6	2.09 ± 0.19	7.7 ± 0.15
LA 1586	L.p.	very small	4.75 ± 0.79	6.47 ± 0.72	19.69 ± 1.10	0.89 ± 0.19	161 ± 20	36.6 ± 4.4	2.07 ± 0.05	8.6 ± 1.2
LA 1593	L.p.	very small	4.96 ± 0.53	6.40 ± 0.40	18.04 ± 0.71	0.89 ± 0.09	143 ± 12	28.5 ± 3.4	1.99 ± 0.02	7.1 ± 0.8
LA 1230	L.e.	very large	3.11 ± 0.40	3.00 ± 0.43	5.16 ± 0.56	0.46 ± 0.06	70 ± 3	15.2 ± 1.2	1.35 ± 0.10	4.2 ± 0.18
LA1247	L.e.	very small	4.49 ± 0.54	4.59 ± 0.40	8.93 ± 0.74	0.55 ± 0.09	96 ± 5	21.8 ± 3.7	1.62 ± 0.06	5.3 ± 0.13
LA1315	L.e.	very small	5.79 ± 0.23	6.26 ± 0.32	10.07 ± 1.89	0.66 ± 0.11	143 ± 11	26.5 ± 2.5	1.72 ± 0.16	6.3 ± 0.35
LA 1385	L.e.	small	5.50 ± 0.44	5.89 ± 0.65	8.23 ± 0.41	0.66 ± 0.05	130 ± 22	22.5 ± 3.3	1.63 ± 0.05	4.7 ± 0.41
LA 1455	L.e.	small	5.04 ± 0.44	5.79 ± 0.61	8.11 ± 1.32	1.00 ± 0.16	123 ± 22	28.6 ± 2.4	1.41 ± 0.10	5.0 ± 0.41
LA 1456	L.e.	very small	5.59 ± 0.43	6.08 ± 0.40	8.52 ± 1.35	1.15 ± 0.09	134 ± 14	26.2 ± 4.3	0.99 ± 0.02	4.4 ± 0.10
Nagcarlan	L.e.	very large	3.54 ± 0.33	3.75 ± 0.42	7.24 ± 1.66	0.70 ± 0.15	88 ± 5	20.8 ± 4.1	1.70 ± 0.17	4.7 ± 0.26
Walter	L.e.	very large	3.61 ± 0.37	2.96 ± 0.41	4.15 ± 0.47	0.36 ± 0.04	63 ± 4	12.1 ± 1.9	1.53 ± 0.13	4.7 ± 0.30
UC204A	L.e.	large	3.56 ± 0.45	2.80 ± 0.28	5.83 ± 0.95	0.42 ± 0.05	63 ± 1	12.3 ± 2.7	1.72 ± 0.15	3.7 ± 0.18
FMTT 33	L.e.	very large	3.55 ± 0.37	3.36 ± 0.34	5.09 ± 0.66	0.44 ± 0.07	72 ± 1	14.2 ± 2.5	1.62 ± 0.14	4.6 ± 0.09
T4065 (*hp*, *og*^{c})	L.e.	very large	2.42 ± 0.54	2.70 ± 0.28	10.64 ± 1.36	0.56 ± 0.03	63 ± 4	18.0 ± 1.7	2.16 ± 0.17	4.6 ± 0.06
T5019 (*hp*, *dg*)	L.e.	very large	3.40 ± 0.69	3.91 ± 0.37	9.30 ± 1.30	1.15 ± 0.14	87 ± 3	17.5 ± 2.6	1.81 ± 0.19	4.2 ± 0.21
CLN1314G (*B*)	L.e.	very large	2.86 ± 0.80	3.23 ± 0.32	1.18 ± 0.04	3.73 ± 0.28	68 ± 3	13.4 ± 2.6	0.73 ± 0.02	4.2 ± 0.14
97L63 (*B*)	L.e.	large	1.77 ± 1.09	2.53 ± 0.16	0.28 ± 0.08	3.38 ± 0.33	56 ± 4	12.9 ± 2.8	0.47 ± 0.03	4.2 ± 0.09
Waller-Duncan			1.36	1.16	2.68	0.29	29	5.3	0.27	1.0
Mean of all entries			3.97 ± 0.08	4.11 ± 0.09	7.21 ± 0.31	0.73 ± 0.05	90 ± 2	19.1 ± 0.6	1.55 ± 0.04	4.8 ± 0.07

Table 2. Capsicum accessions evaluated for antioxidant activity, antioxidants, and dry matter, AVRDC-the World Vegetable Center, Taiwan. Values are means calculated on a dry weight basis averaged over two years (± SE).

Entry	ILP	Cap-santhin	Zeaxanthin	Lutein	β-crypto-xanthin	β-carotene	Phenolics	Ascorbic acid	α-tocopherol	Dry matter
	TE (µmol•g^{-1})	(µg•g^{-1})					(mg•g^{-1})		(µg•g^{-1})	%
Perennial HDV	16.3 ± 3.6	462 ± 41	157 ± 27	52 ± 4	37 ± 4	80 ± 10	18.0 ± 1.6	6.5 ± 1.0	419 ± 16	29 ± 2
Nose Gay	21.1 ± 1.0	257 ± 17	28 ± 8	32 ± 3	12 ± 1	33 ± 5	19.7 ± 1.7	9.4 ± 1.3	57 ± 6	22 ± 1
P484/76	28.4 ± 2.7	0	0	133 ± 10	14 ± 4	61 ± 7	26.3 ± 2.1	9.4 ± 1.2	502 ± 98	10 ± 0.4
CAP178/77	61.8 ± 5.8	714 ± 61	155 ± 15	95 ± 8	81± 7	129 ± 17	37.6 ± 3.1	18.8 ± 1.9	551 ± 61	11 ± 0.3
P126/79	13.0 ± 0.7	398 ± 83	121 ± 14	58 ± 10	31 ± 3	41 ± 7	12.3 ± 0.8	2.6 ± 0.2	349 ± 31	36 ± 2
PI196181	20.4 ± 1.6	858 ± 172	114 ± 20	108 ± 23	73 ± 11	78 ± 4	19.5 ± 1.3	5.5 ± 0.5	507 ± 40	20 ± 0.2
PI124540	29.1 ± 1.8	749 ± 110	142 ± 30	90 ± 12	59 ± 11	90 ± 17	26.3 ± 2.3	12.8 ± 1.4	635 ± 44	20 ± 2
Mexico Serrano	24.2 ± 2.4	374 ± 55	47 ± 4	44 ± 6	24 ± 3	66 ± 11	22.5 ± 0.6	8.1 ± 2.1	306 ± 19	15 ± 0.3
Agronomico 9 Lines	53.0 ± 9.7	614 ± 168	136 ± 45	82 ± 24	65 ± 22	102 ± 31	32.4 ± 5.7	16.7 ± 3.6	423 ± 73	10 ± 0.3
Roque Mulato Poblano	32.1 ± 3.3	555 ± 123	264 ± 76	73 ± 16	58 ± 13	91 ± 12	28.2 ± 2.2	14.7 ± 1.1	466 ± 64	18 ± 1
Verdano Poblano TY	42.1 ± 2.8	1333 ± 349	207 ± 65	173 ± 50	103 ± 37	275 ± 70	32.6 ± 0.8	17.9 ± 1.1	859 ± 106	14 ± 0.2
Guajillo Ancho	38.0 ± 4.2	1585 ± 280	213 ± 47	210 ± 39	128 ± 38	292 ± 73	29.5 ± 3.8	14.8 ± 2.4	759 ± 181	20 ± 2
Prapadaeng	17.2 ± 0.8	570 ± 71	209 ± 43	70 ± 9	52 ± 4	56 ± 9	17.3 ± 1.6	7.2 ± 0.6	434 ± 18	25 ± 0.5
Lueng 4	24.3 ± 2.4	0	30 ± 23	189 ± 17	62 ± 13	111 ± 22	24.7 ± 1.8	10.6 ± 1.0	405 ± 15	19 ± 0.7
CAB	47.8 ± 1.6	416 ± 119	47 ± 11	53 ± 16	45 ± 10	84 ± 23	33.8 ± 1.3	17.1 ± 1.0	253 ± 29	10 ± 1
Waller-Duncan	6.7	442	97	63	42	96	4.4	2.9	206	4
Mean of all entries	27.3 ± 0.9	552 ± 30	115 ± 7	83 ± 4	53 ± 3	98 ± 6	23.5 ± 0.5	9.0 ± 0.4	455 ± 15	19 ± 0.5

of the red-fruited entries. Entry means over years for total phenolics ranged from 12.3-37.6 mg·g^{-1}. CAP178/77, Agronomico 9, Nacional AG-506, and CAB ranked among the top for both ascorbic acid and total phenolics. We found wide variation among entry means for α-tocopherol content (75-882 µg·g^{-1}) and Verdano Poblano and Guajillo Ancho were outstanding for high α-tocopherol content.

DISCUSSION

Genetic improvement of tomato for AOA and specific AO is possible given the large genetic variation present among tomato entries. However, entries outstanding for AOA and most AO in this study were exclusively *L. pimpinellifolium* or small-fruited *L. esculentum* and the medium or large-fruited entries in this study showed average or below average AOA. Although information is lacking on the improvement status of many entries, it is likely that the large-fruited entries were developed in plant breeding programs while *L. pimpinellifolium* and small-fruited *L. esculentum* are non-bred germplasm. Further work is required to determine if lower AOA of the larger, and presumably bred lines, is a consequence of increased fruit size or the result of selection against certain AO, especially phenolics, in the course of domestication/ plant breeding.

Among AO, total phenolics demonstrated the largest positive correlations with both ARP and ILP (r=0.90** and r=0.93**, respectively). Tomato breeders have given little attention to the phenolics content of tomato fruit but our study suggests that genetic improvement to increase total phenolics content is a worthy objective. All AO in this study, including total phenolics, were negatively correlated with fruit size (r= -0.72), suggesting that development of cultivars of commercial fruit size with high phenolics content may be difficult.

Ample genetic diversity exists within *C. annuum* for AOA and the AO evaluated in this study and genetic improvement of these traits is possible. *C. annuum* cultivar market types are highly segmented according to intended use, pod type (shape, size, and color), level of pungency, regional preferences and origins (DeWitt and Bosland, 1996). Hot pepper and sweet pepper constitute the major division of *C. annuum* (DeWitt and Bosland, 1996). Our data point to differences between pungent and non-pungent *C. annuum* in content of some AO although comparisons are complicated by the great difference in dry matter content between the two groups (22% versus 12% for pungent and non-pungent *C. annuum*, respectively). On a DW basis, non-pungent *C. annuum* as a group, contained higher concentrations of ascorbic acid (65%), total phenolics (36%), α-tocopherol (11%), and ß-cryptoxanthin (36%) compared to the group mean of the pungent accessions. Among the red-fruited entries, 'Verdano Poblano' and 'Guajillo Ancho', both 'Ancho' types originating from Mexico, ranked among the best entries for content of almost every AO, including individual carotenoids, ascorbic acid, α-tocopherol, and total phenolics. These two accessions are of particular interest and additional accessions of the Ancho/Poblano type should be investigated for their nutritional qualities.

Greater realization of the importance of phytochemicals in promotion of human health has prompted the broadening of dietary recommendations to include greater daily intake of a wider range of vegetables and fruit. This will be difficult in the tropics and subtropics where vegetable supplies are both highly seasonal and daily per capita availability fall drastically below the 200g recommended by AVRDC-the World Vegetable Center (Ali and Tsou, 1997). Increased phytochemical intake in the tropics requires multiple tactics, including production of tropically adapted cultivars with higher densities of micronutrients and AO; overcoming seasonality of vegetable production so affordable vegetables are available to poor consumers throughout the year; diversification of diets to include antioxidant-rich vegetable species.

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